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(54) Title: SYNTHETIC PEPTIDES AND PSEUDOPEPTIDES HAVING OSTEOGENIC ACTIVITY AND PHARMACEUTICAL COMPOSITIONS CONTAINING THE SAME

(57) Abstract

The invention relates to synthetic pseudopeptide derivatives of osteogenic growth polypeptide (OGP) and OGP (10-14) which may be linear or cyclic, and which are capable of enhancing bone cell proliferation and bone formation. Further, the present invention relates to pharmaceutical composition comprising as active ingredient at least one pseudopeptide derivative of the invention and to the use of these pseudopeptide derivatives in the preparation of a pharmaceutical composition for stimulating the formation of osteoblastic or fibroblastic cells, enhancing bone formation in osteopenic pathological conditions, repairing fractures, healing wounds, grafting of intraosseous implants, reversing bone loss in osteoporosis and other conditions requiring enhanced bone cells formation.

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SYNTHETIC PEPTIDES AND PSEUDOPEPTIDES HAVING OSTEOGENIC ACTIVITY AND PHARMACEUTICAL COMPOSITIONS CONTAINING THE SAME

FIELD OF THE INVENTION

The present invention relates to synthetic pseudopeptide derivatives of OGP and OGP(10-14) which are capable of enhancing bone cell proliferation and bone formation.

BACKGROUND OF THE INVENTION

It has been established that regenerating bone marrow induces an osteogenic response in distant skeletal sites and that this activity is mediated by factors released into the circulation by the healing tissue [(Bab I., *et al.* (1985) Calcif. Tissue Int. 37:551; Foldes, J., *et al.* (1989) J. Bone Min. Res. 4:643; Einhorn, T.A., *et al.* (1990) J. Bone Joint Surg. Am. 72:1374; Gazit D., *et al.* (1990) Endocrinology 126:2607; Mueller, M., *et al.* (1991) J. Bone Min. Res. 6:401]. One of these factors, a 14-amino acid osteogenic growth polypeptide (OGP), identical with the C-terminus of histone H4, has been recently identified in the regenerating bone marrow [Bab, I., *et al.* (1992) EMBO J. 11:1867; EP-A-0 384 731] and in human serum [Greenberg, Z *et al* (1995) J. Clin. Endocrinol. Metab 80:2330].

Synthetic osteogenic growth polypeptide, identical in structure with the native molecule, has been shown to be a potent stimulator of proliferation of osteoblastic and fibroblastic cells *in vitro*. This synthetic polypeptide also stimulates osteoblastic cell alkaline phosphatase activity. When injected *in vivo* to rats, at very small doses, the synthetic osteogenic growth polypeptide increases bone formation and trabecular bone mass [Bab, I., *et al* (1992) EMBO J. 11:1867].

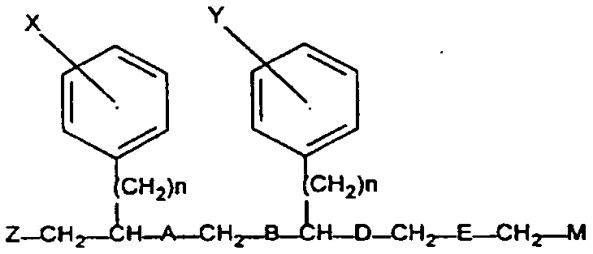
Since the OGP molecule is too large for effective oral administration, it is of therapeutic importance to identify peptides, shorter than the full length OGP, that retain the OGP activity and can be modified into a stable preparation, suitable for the oral treatment of several pathological conditions, particularly conditions involving loss of bone tissue. Indeed, it was shown that the C-terminal penta-peptide of OGP, Try-Gly-Phe-Gly-Gly[OGP(10-14)], retains the full OGP-like proliferative activity *in vitro* and osteogenic effect *in vivo* [WO94/20529 corresponding to Israel Patent Application No. 104954]. Due to its small size, this

penta-peptide provides a useful basis for the design of further OGP analogs with improved activity, stability and bioavailability.

In search for yet improved osteogenically active substances, the inventors have now found novel, synthetic pseudopeptide derivatives of OGP and OGP(10-14), which are the subject of the present application.

BRIEF DESCRIPTION OF THE INVENTION

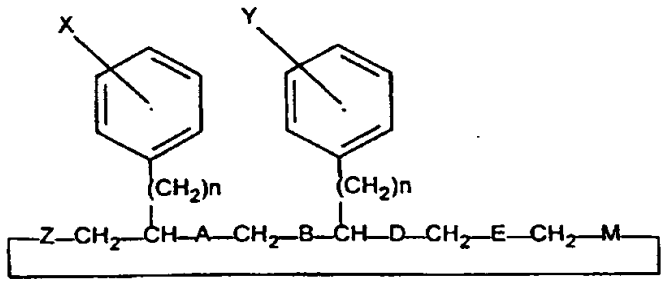
The present invention relates to pseudopeptidic osteogenic growth polypeptide (OGP) analogs having the general formula:



(I)

wherein the substituents are as hereafter defined.

The invention also relates to cyclic peptidic or pseudopeptidic OGP analogs having the general formula:



(II)

wherein the substituents are as hereafter defined.

The invention also relates to pharmaceutical compositions comprising as active ingredients the compounds of formulae (I) and/or (II).

DESCRIPTION OF THE FIGURES

Figure 1 shows the linear regression of proliferative activity of OGP between osteoblastic MC3T3E1 and fibroblastic NIH3T3 cells.

Figure 2 shows the dose-response relationship of proliferative activity of cyclic OGP analogs in cultures of osteoblastic MC3T3E1 cells as compared with negative control cultures not treated with any peptide (C) and positive control cultures treated with synthetic OGP(1-14). Data are mean±SE obtained in three culture wells per condition.

Figure 3 shows the dose-response relationship of proliferative activity of constrained OGP analogs with substitution of the peptide bond between Leu^9 and Tyr^{10} in cultures of osteoblastic MC3T3E1 (A) and fibroblastic NIH3T3 (B) cells as compared with negative control cultures not treated with any peptide (C) and positive control cultures treated with synthetic OGP(1-14) or OGP(10-14). Data are mean±SE obtained in three culture wells per condition.

Figure 4 shows the dose-response relationship of proliferative activity of photoreactive OGP analogs in cultures of osteoblastic MC3T3E1 cells as compared with negative control cultures not treated with any peptide (C) and positive control cultures treated with synthetic OGP(1-14) or OGP(10-14). A-[Bpa^{12}]OGP(10-14); B-±Nα-biotinylcaproyl-[Bpa^{12}]OGP(10-14) and positive controls. Data are mean±SE obtained in three culture wells per condition.

Figure 5 shows the effect of synthetic OGP analogs on reversal of trabecular bone loss in proximal tibial metaphysis of ovariectomized mice. Data are mean± SE obtained in eight mice per group.

Figure 6 shows the effect of OGP analogs on reversal of reduction in osteoprogenitor cells in bone marrow of ovariectomized rats as reflected in number of bone marrow derived *in vitro* osteoblastic colonies. Data are mean±SE obtained in five rats per group.

Figure 7 shows the dose-response inhibition of stimulatory effect of optimal OGP(1-14) dose on osteoblastic MC3T3E1 cell as compared with negative control cultures not treated with any peptide (C). All other cultures were

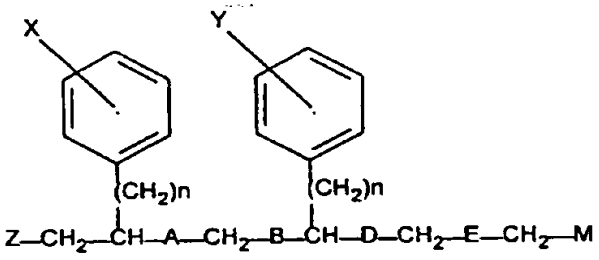
treated with 10^{-13}M synthetic OGP(1-14) and the indicated dose of antagonist. Data are mean±SE obtained in three culture wells per condition.

Figure 8 shows the dose-response relationship of anti-proliferative activity of OGP antagonists in cultures of osteoblastic MC3T3E1 cells as compared with negative control cultures not treated with any peptide (C) and positive control cultures treated with synthetic OGP(1-14). Data are mean±SE obtained in three culture wells per condition.

DETAILED DESCRIPTION OF THE INVENTION

Osteogenic growth polypeptide (OGP) is a 14-residue polypeptide identified from regenerating bone marrow which has been shown to stimulate the proliferation and alkaline phosphatase activity of osteoblastic and fibroblastic cells *in vitro* and to increase bone formation and trabecular bone mass in rats when injected *in vivo*. In addition, shorter, tetra- and pentapeptides, derived from the C-terminal of OGP have been identified, which retain the OGP activity. Naturally, such short peptides may have advantages as therapeutic agents, being smaller molecules than the native or synthetic full length OGP. The present invention is concerned with various modifications of these peptides, which may be of major interest as potent agonists and antagonists of OGP.

The present invention thus relates to pseudopeptidic osteogenic growth polypeptide (OGP) analogs having the general formula:



(I)

wherein

A, B, D and E, which may be the same or different, represent CONH, CH_2NH, CH_2S, CH_2O, NHCO, $N(CH_3)CO$, $(CH_2)_2$, CH=CH, $C(O)CH_2$, CH_2SO or C(O)O,

M represents C(O)OH, CH_2OH, $C(O)NH_2$, $C(O)OCH_3$, CH_2OCH_3, H, $C(O)NHCH_3$, or $C(O)N(CH_3)_2$,

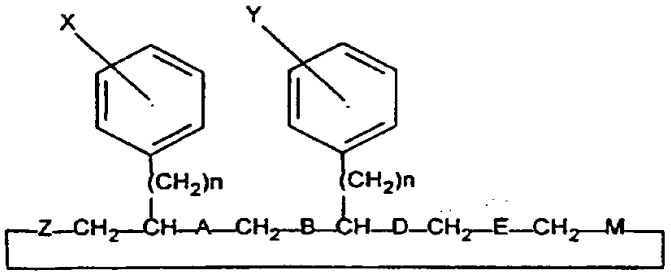
Z represents NH_2, H, $NHCH_3$, $N(CH_3)_2$, OH, SH, OCH_3, SCH_3. C(O)OH, $C(O)NH_2$, $C(O)OCH_3$, $C(O)NHCH_3$ or $C(O)N(CH_3)_2$,

n and m each represent an integer of 1 to 6,

X and Y, if in the ortho or para positions, each represent OH, OCH_3, F, Cl, Br, CF_3, CN, NO_2, NH_2, $NHCH_3$, $N(CH_3)_2$, SH, SCH_3, CH_2OH, $NHC(O)CH_3$, C(O)OH, $C(O)OCH_3$, $C(O)NH_2$, $C(O)NHCH_3$, $C(O)N(CH_3)_2$, or CH_3, and

Y, if in the para or meta positions, represents $C(O)C_6H_5$, $C(O)CH_3$, C_6H_5, $CH_2C_6H_5$, and, if in the ortho or para positions can additionally represent $C(O)C_6H_5$, $C(O)CH_3$, C_6H_5, $CH_2C_6H_5$, CH_2CH_3, $CH(CH_3)_2$, or C_6H_{11}.

The invention also relates to cyclic peptidic or pseudopeptidic OGP analogs having the general formula:



(II)

wherein Z — M represent NHC(O), C(O)NH, CH_2NH, NH_2CH_2, $N(CH_3)C(O)$, $C(O)N(CH_3)$, C(O)O, OC(O), OR $(CH_2)_l$ where l is an integer of from 2 to 6 and A, B, D, E, n, m, X and Y are as hereinbefore defined.

A particular pseudopeptidic OGP analog of formula (I) is desaminoTyr-Gly-Phe-Gly-Gly (referred to in the following Examples as desamino[Tyr^{10}]OGP(10-14)), demonstrating a retention of approximately 70% OGP-like activity (Table 1, analog 4), indicating the minor role of the α-amino group in the OGP activity. Furthermore, *in vivo* effects of this analog (Figure 5,6) were either similar or superior to the parent oligopeptides, namely, OGP(1-14) and OGP(10-14).

Other particular pseudopeptidic OGP analogs of formula (I) are desaminoTyr-Gly-N(CH_3)-CH($CH_2C_6H_5$)-C(O)-Gly-Gly (referred to in the following Examples as desamino[TyR^{10},N(Me)-Phe^{12}]OGP(10-14)), desaminoCH($CH_2C_6H_5OH$)-CH_2-Gly-Phe-Gly-Gly (referred to in the following Examples as desamino[Tyr$^{10}\psi$(CH_2NH)-Gly^{11}]OGP(10-14)), desaminoTyr-NH-CH_2-CH_2-Phe-Gly-Gly (referred to in the following Examples as desamino[Tyr^{10},Gly$^{11}\psi$(CH_2NH)Phe^{12}]OGP(10-14)), desaminoTyr-Gly-NH-CH($CH_2C_6H_5$)-CH_2-Gly-Gly (referred to in the following Examples as desamino[Tyr^{10},Phe$^{12}\psi$(CH_2NH)Gly^{13}]OGP(10-14)), desaminoTyr-Gly-Phe-NH-CH_2-CH_2-Gly (referred to in the following Examples as desamino[Tyr^{10},Gly$^{13}\psi$(CH_2NH)Gly^{14}]OGP(10-14)), desaminoTyr-Gly-Phe-NH-CH_2-CH_2-CH_2-CH_2-C(O)-OH (referred to in the following Examples as desamino[Tyr^{10},Gly$^{13}\psi$(CH_2)$_2$Gly^{14}]OGP(10-14)), Tyr-Gly-NH-CH($CH_2C_6H_4$-(C(O)-C_6H_5))-C(O)-Gly-Gly (referred to in the following Examples as [Bpa^{12}]OGP(10-14)), Tyr(m-I)-Gly-NH-CH($CH_2C_6H_4$(C(O)C_6H_5))C(O)-Gly-Gly (referred to in the following Examples as [Tyr^{10}(m-I),Bpa^{12}]OGP(10-14)) and Nα-biotinylcaproyl[Bpa^{12}]OGP(10-14), all showing *in vitro* potency, relative to that of OGP, of above 0.5, similar or improved activity compared to desamino[Tyr^{10}]OGP(10-14) (Tables 5,6)

A particular cyclic peptidic OGP analog of formula (II) is:

Tyr—Gly—Phe—Gly—Gly (refered to in the following Examples as c[Tyr-Gly-Phe-Gly-Gly]. This cyclization is another mode to rigidify the OGP(10-14) structure. As can be seen in Figure 2 this rigidification preserves the OGP-like *in vitro* activity. In addition, Figure 6 exhibits an improved *in vivo* activity of c[Tyr-Gly-Phe-Gly-Gly] over OGP(10-14). Also, introduction of D-amino acids into this cyclic peptide, as, for example D-Tyr—Gly—D—Phe—Gly—Gly (referred to in the following Examples as c[D-Tyr-Gly-D-Phe-Gly-Gly]) resulted in a peptide which had a considerable level of proliferative activity.

Other particular cyclic peptidic or pseudopeptidic OGP analogs of formula (II) are:

Gly—Gly—Phe—Gly—Tyr (referred to in the following Examples as c[Gly-Gly-Phe-Gly-Tyr]), and Gly—Gly—D—Phe—Gly—D—Tyr (referred to in the following Examples as c[Gly-Gly-D-Phe-Gly-D-Tyr]) demonstrating a similar or slightly improved *in vitro* activity (Table 5). Interestingly, the retro analog, in which the sequence of the amino acids was reversed, retained a full OGP-like proliferative activity,

suggesting the irrelevance of amide bond direction in the backbone. This observation is also displayed in the constrained, linear pseudopeptides, as shown in Table 5. The improved efficacy of the present constrained analogs might be due to increased resistance to peptidase degradation and longer persistence in circulation or increased potency and bioavailability, as described in the following Examples.

In addition, the invention relates to peptidic and pseudopeptidic osteogenic growth polypeptide antagonists such as, for example, Leu-N(CH_3)-CH($CH_2C_6H_4$(OH))-C(O)-Gly-Phe-Gly-Gly ([N(CH_3)-Tyr^{10}]OGP(9-14)) as herein defined) and Tyr-Gly-Phe-Gly-Asp ([Asp^{14}]OGP(10-14)) referred to in the following Examples as [N-(CH_3)-Tyr^{10}]OGP(9-14)). As can be seen in Figure 7, the present antagonists have an inhibitory effect at low doses on stimulation by an optimal OGP(10-14) dose on osteoblastic MC3T3 E1 cells. Moreover, in the absence of exogenous OGP(10-14) the present antagonists demonstrate an anti-proliferative activity in the MC3T3 E1 cells. Nevertheless, a reversal effect is obtained at higher doses, thus showing a dose-dependent response to [N(CH_3)Tyr^{10}]OGP(9-14) and [Asp^{14}]OGP(10-14). These antagonists may be useful in the treatment of conditions characterized by excess OGP.

The invention also relates to pharmaceutical compositions comprising as active ingredient a pseudopeptide of formula (I), optionally with a pharmaceutically acceptable carrier. Particularly preferred are pharmaceutical compositions in which said pseudopeptide is desamino[Tyr^{10}]OGP(10-14).

A further aspect the invention relates to pharmaceutical compositions comprising as active ingredient a cyclic peptide or pseudopeptide of formula (II), optionally with a pharmaceutically acceptable carrier. Pharmaceutical compositions in which said cyclic peptide is c[Tyr-Gly-Phe-Gly-Gly] are preferred.

The pseudopeptides of formula (I) and cyclic peptides or pseudopeptides of formula (II) may be particularly useful in the preparation of pharmaceutical compositions for stimulating the formation of osteoblastic or fibroblastic cells, enhancing bone formation in osteopenic pathological conditions, repairing fractures, healing wounds, grafting of intraosseous implants, reversing bone loss in osteoporosis and other conditions requiring enhanced bone cells formation.

EXAMPLES

Materials and Methods

General

Boc-amino acids were purchased from either Bachem, California or prepared with di-tert.butyl dicarbonate by conventional procedure [Morodor, L., *et al* (1976) Physiol. Chem. 357:1651]. All chemicals were purchased from Aldrich Chemical Co., Fluka Chemie AG or Pierce Chemical Co. and were of analytical grade. Peptidic and pseudopeptidic OGP analogs were treated with liquid HF in an all-Teflon apparatus (Protein Research Foundation, Osaka, Japan). Thin layer chromatography (TLC) was performed on precoated silica gel plates 60F-254 (E. Merck, Darmstadt, FRG) in the following solvent systems (all v/v): (i) 1-BuOH/AcOH/H_2O (4:1:1); (ii) 1-BuOH/AcOH/EtOAc/H_2O (5:1:3:1); (iii) $CHCl_3$/MeOH/AcOH (9:3:1). Analogs were visualized by UV light and/or ninhydrine staining. Analytical and semipreparative HPLC separations were performed on a Merck Hitachi 655A-11 apparatus, equipped with 655A Variable Wavelength and L-5000 LC Controller, D-2000 Chromato-Integrator and an AS-2000 Autosampler injector. Light absorbance was recorded at 220 nm. A reverse phase Lichrospher 100 C-18 column was used for all analytical applications. The crude OGP analogs were purified on a μBondpark C-18, 19X150 mm or a Vydac Protein & Peptide C-18 column employing acetonitrile containing 0.1% (v/v) trifluoroacetic acid in water. Flow rates were 1 ml/min for the analytical column and 6 ml/min for the semipreparative column.

Synthesis of OGP analogs

Unless otherwise indicated, the peptidic or pseudopeptidic OGP analogs of this invention were prepared manually on a Milligen 504 Synthesizer or automatically using a 401A Applied Biosystem Peptide Synthesizer. Boc-Amino acids were assembled on a PAM resin, Merrifield resin, Oxime resin or MBHA resin [Merrifield (1969) Adv. Enzymol. 32:221]. The fully assembled analog was removed from the resin either by ammonolysis or the HF procedure.

The preparations were evaluated for purity using analytical HPLC (Vydac C-18 column) and were shown to be more than 95% pure. The molecular weight of the analogs was verified by Fast Atom Bombardment Mass Spectroscopy (FAB-MS). When applicable the analogs were subjected to amino acid analysis.

Introduction of C-terminal modifications

C-terminal modifications were introduced by coupling an active ester with the corresponding amine component either during cleavage from the resin or later in solution [Stewart, J.M., Young, J.D., (1984) In: Solid Phase Peptide Synthesis. Pierce Chemical Co.: Rockford, IL, pp. 1-75].

Preparation of cyclic analogs

N- to C-terminal cyclization was carried out in a low concentration (0.008 M) solution of the corresponding linear peptide in amine-free dimethylformamide (DMF) at 0°C. The coupling agent was diphenol-phosphoryl azide (1.5 equivalent) [Lender, A., *et al* (1993) Int. J. Peptide Protein Res., 42:509]. Upon completion of the reaction the solvent was removed by evaporation and the cyclic analog purified by reverse phase HPLC.

N-terminal to side chain cyclization was carried out with the peptide chain assembled on an Oxime resin. After the removal of the N-terminal protecting group the Oxime resin-bound peptide was subjected to a cyclization-cleavage step [Nishino, N., *et al* (1992) Tetrahedron Letters, 33:1479].

Preparation of analogs with N-methylated Boc-amino acids

The Boc-amino acid used for preparation of the corresponding analogs was dissolved in dry methyl iodide supplemented tetrahydrofurane. N-methylation was induced by NaH. The solvent was removed *in vacuuo* and the crude product purified by flash column chromatography eluted with EtOAc-petroleum ether [Cheung, S.T. and Benoiton, N.L., (1977) Can. J. Chem., 55:906].

N-terminal acetylation

Following N-terminal deprotection and prior to cleavage, the resin bound peptide was treated with acetyl hydride and N,N-diisopropylethylamine (DIEA).

Introduction of reduced amide bonds

The introduction of the $\psi(CH_2NH)$ peptide bond isostere into the corresponding peptides was accomplished by solid phase reaction of the N-terminal amino group of the resin bound peptide with the requisite Boc-protected amino acid aldehyde in the presence of sodium cyanoborohydride in DMF containing 1% AcOH [Sasaki, Y. and Coy, D.H., (1987) Peptides, 8:119]. The corresponding aldehydes were

prepared by $LiAlH_4$ reduction [Fehrentz, J.-A. and Castro B., (1983) Synthesis, pp. 676-678] of their *N,O*-dimethyl hydroxamates [Hocart, S.J., *et al* (1988) J. Med. Chem. 31:1820].

Preparation of Nα-Biotinylcaproyl-OGP(10-14)

The purified OGP(10-14) was dissolved in dry DMF containing an equivalent of DIEA and biotin reagent. The reaction mixture was adjusted to pH 8.5 with DIEA. The crude product was neutralized with AcOH and the solvents removed *in vacuuo* [Wilchek, M. and Bayer, E.A., (1990) Methods Enzymol 184:5].

Proliferation assay

The effect of OGP analogs on osteoblastic MC3T3 E1 and fibroblastic NIH 3T3 cell proliferation was measured as before [Bab, I., *et al* (1992) EMBO J. 11:1867]. Some of the analogs were subjected to a dose response analysis. Otherwise the analog concentration was 10^{-13}M and 10^{-11}M in the MC3T3 E1 and NIH3T3 cell cultures, respectively. The mean cell number in triplicate culture wells was expressed as percent of a positive control triplicate dosed with OGP(1-14). Experiments testing one dose per cell line were repeated at least four times and the activity of individual analogs expressed as the mean of results and 95% confidence limit obtained in these repetitive experiments.

Osteogenic effect of OGP analogs in ovariectomized mice

Thirty two female C57Bl/6 mice weighing 25 gm underwent conventional bilateral ovariectomy (OVX). Additional eight control animals were subjected to sham OVX: the anterior abdominal wall was opened and the ovaries exposed but left intact. All animals were left untreated for 30 days. The OVX animals were then divided into four groups each consisting of eight mice. All animals were injected subcutaneously in the nape daily for six weeks with the following solutions: One group was given OGP(1-14), 30 ng/day/mouse. A second group received OGP(10-14), 10 ng/day/mouse. A third group was given desamino[Tyr^{10}]OGP(10-14). All compounds were dissolved in phosphate buffered saline (PBS). An additional control OVX group was given the PBS solvent only. One day after termination of treatment the animals were killed and the tibial bones separated, fixed in phosphate buffered formalin and subjected to conventional decalcified histological processing. Sections through the midsagital region of the tibia were stained with Masson trichrome. Bone volume was determined in the secondary spongiosa of the proximal metaphysis in two sections 200-300 μm apart from each other in one tibia

from each animal using an automated computerized image analyzer. The value for each animal was the mean reading from the two sections.

Effect of OGP analogs on the number of bone marrow derived osteoblastic colonies from ovariectomized rats

Twenty five female Sabra rats weighing 250 g each were subjected to bilateral ovariectomy (OVX). Additional five control animals underwent sham OVX. All animals were left untreated for 30 days. Then the OVX animals were divided into five groups, each consisting of five rats. All animals were injected subcutaneously in the nape daily for eight weeks with following solutions: One group was given OGP(10-14), 100 ng/day/rat. A second group was given desamino[Tyr^{10}]OGP(10-14), 100 ng/day/rat. A third group was given c(Tyr-Gly-Phe-Gly-Gly), 100 ng/day/rat. The fourth group was given retro OGP (Gly-Gly-Phe-Gly-Tyr-Leu-Thr-Arg-Gly-Gln-Arg-Lys-Leu-Ala), 300 ng/day/rat. All compounds were dissolved in PBS. An additional control OVX group was given the PBS solvent only. After termination of treatment the animals were killed and the femoral and tibial bone marrow from both posterior limbs was pooled and transferred to alpha minimal essential medium (αMEM). Bone marrow cell cultures were set in 35 mm dishes, 10 dishes per animal, as described previously [Rickard, D.J., *et al* (1994) Biology, 161:218] The total number of fibroblastic colonies (CFU-f) formed was determined after three weeks in culture. Immediately after, the CFU-f cultures were stained for alkaline phosphates and co-stained for mineral with alizarin-red-S. The alizarin-red-S positive colonies were considered osteoblastic. Their frequency was expressed as their percentage of the total numbers of colonies. The value for each animal was calculated as the mean percentage obtained in the 10 dishes.

Results

The proliferative activity of synthetic OGP analogs is shown in Tables 1-6. There was a very high correlation of the proliferative activity of the analogs between the osteoblastic MC3T3 E1 and fibroblastic NIH3T3 cells (Figure 1). The scatter plot of the MC3T3 E1/ NIH3T3 relationship (Figure 1) demonstrates three clusters of analogs, namely (i) those with activity higher than 50% compared to OGP(1-14); (ii) those showing less than 50% activity compared to OGP(1-14); and (iii) those that inhibit cell proliferation. Only one analog, desamino[Tyr^{10}]OGP(10-14)-OMe, could not be assigned to one cluster in the sense that it showed slightly more than 50% activity in the MC3T3 E1 cells and less than 50% activity in the NIH3T3 cells (Table 1, analog 8). The activity of few analogs, [Bpa^{12}]OGP(10-14) (Table 7,

analog 2), [Tyr^{10}(m-I), Bpa^{12}]OGP(10-14) (Table 7, analog 3), [Pro^{11}]OGP(10-14) ((Table 5, analog 2), desamino[Tyr$^{10}\psi$(CH$_2$NH)Gly^{11}]OGP(10-14) (Table 6, analog 2), desamino[Tyr^{10},Gly$^{13}\psi$(CH$_2$NH)Gly^{14}]OGP(10-14) (Table 6, analog 5), desamino[Tyr^{10},Gly$^{13}\psi$(CH$_2$)$_2$Gly^{14}]OGP(10-14) (Table 6, analog 6), c(Tyr-Gly-Phe-Gly-Gly) (Table 5, analog 7), c(Gly-Gly-Phe-Gly-Tyr) (Table 5, analog 9) and c(Gly-Gly-D-Phe-Gly-D-Tyr) (Table 5, analog 11), was similar to that of OGP(1-14) or even higher. The activity of Nα-Ac-OGP(12-14) (Table 1, analog 3), desamino[Tyr^{10}]OGP(10-13)NH(CH$_2$)$_2$_OMe (Table 1, analog 12), [Ala^{11}]OGP(11-14) (Table 2, analog 2), [Gly$^{13}\psi$(CH$_2$)$_2$Gly^{12}]OGP(11-14) (Table 6, analog 7), c(βAla-Tyr-Gly-Phe-Gly-Asp)-OH (Table 5, analog 18) and c(γAbu-Tyr-Gly-Phe-Gly-Asp) (Table 5, analog 19), was essentially nil. Some of the analogs were subjected to a dose-response analysis in the MC3T3E1 and NIH3T3 cell proliferation assays. The resulting biphasic dose-response curve was similar to that of OGP(1-14) and OGP(10-14) [Bab, I., *et al.* (1992) EMBO J. 11:1867; Greenberg, Z., *et al* (1993) Biochim Biophys Acta 1178:273] with a dose-dependent stimulation at low concentrations followed by a dose-dependent reversal of this stimulation at high doses. The peak response in the MC3T3 E1 and NIH3T3 cells was at 10^{-13}M and 10^{-11}M peptide concentration, respectively (Figures 2-4).

Amino terminal group analysis indicated that the α-amine group has only_a small role in the OGP activity as demonstrated by the retention of approximately 70% OGP-like activity by desamino[Tyr^{10}]OGP(10-14) (Table 1, analog 4). The *in vivo* effects of this analog, namely, the_respective reversal of trabecular bone loss and reduction in osteoprogenitor cells in osteoporotic OVX mice and rats, were either similar or superior to those of OGP(1-14) and OGP (10-14) (Figures 5,6) probably because of increased resistance to degradation by amino peptidases. Removal of Tyr^{10} (Table 1, analog 2; Table 2, analog 2) or its replacement by L-Ala (Table 2, analog 5), D-Ala (Table 2, analog 5), desaminoAla (Table 2, analog 7), Phe (Table 3, analog 2), desaminoPhe (Table 3, analog 3) or (desaminoPhe)$_2$ (Table 3, analog 4) resulted in loss of more than 70% activity.

Table 1. Proliferative activity of OGP(10-14) analogs with modified termini

	Analog	Relative *in vitro* potency (95 confidence limit)	
		MC3T3 E1 cells	NIH 3T3 cells
1	OGP(1-14)	1.00 (standard)	1.00 (standard)
2	Nα-Ac-OGP(11-14)	0.21(0.17-0.25)	0.22(0.17-0.27)
3	Nα-Ac-OGP(12-14)	0.06(0.02-0.11)	0.07(0.03-0.11)
4	desamino[Tyr^{10}]OGP(10-14)	0.77(0.66-0.88)	0.66(0.54-0.78)
5	OGP(11-14)-ol	0.24(0.20-0.29)	0.38(0.35-0.42)
6	desamino[Tyr^{10}]OGP(10-14)-NH_2	0.20(0.05-0.35)	0.16(0.05-0.27)
7	desamino[Tyr^{10}]OGP(10-14)-ol	0.24(0.14-0.34)	0.28(0.14-0.42)
8	desamino[Tyr^{10}]OGP(10-14)-OMe	0.51(0.43-0.59)	0.36(0.29-0.43)
9	desamino[Tyr^{10}]OGP(10-14)-NHMe	0.18(0.06-0.30)	0.16(0.08-0.28)
10	desamino[Tyr^{10}]OGP(10-14)-$N(Me)_2$	0.12(0.08-0.21)	0.16(0.05-0.27)
11	desamino[Tyr^{10}]OGP(10-13)-$NH(CH_2)_2NH_2$	0.18(0.07-0.29)	0.17(0.06-0.28)
12	desamino[Tyr^{10}]OGP(10-13)-$NH(CH_2)_2OMe$	0.03(0.00-0.06)	0.06(0.01-0.11)
13	desamino[Tyr^{10}]OGP(10-13)-NHEt	0.19(0.02-0.36)	0.20(0.11-0.31)

Because of its high *in vitro* and particularly *in vivo* OGP-like activity, the desamino[Tyr^{10}]OGP(10-14) was used as a basis for carboxy terminal modifications and L-and D-Ala scanning. This analysis shows that at least in a linear structure the intact Gly^{14} is essential for a significant_level of mitogenic activity inasmuch as all analogs with carboxy terminal group modifications, except maybe desamino[Tyr^{10}]OGP(10-14)-OMe, lost most the OGP-like activity (Table 1).

The replacement of individual amino acids in both OGP(10-14) and desaminoTyr^{10}(10-14) by L- or D-Ala or even desamination of Gly^{11} resulted in all cases in substantial loss of OGP-like proliferative activity (Tables 2,4). These findings further suggest that in both the MC3T3E1 and NIH3T3 cell systems (i) the aromatic ring of Phe^{12} is essential for a significant level of OGP-like proliferative activity; (ii) the spatial relationship between the phenolic OH group of Tyr^{10} and aromatic ring of Phe^{12}, including the distance between these groups, may be also

important for this activity. In disagreement with the Ala substitution of Gly^{13} is the replacement of this residue by His which has no consequences upon the activity of OGP(10-14) [WO94/20529 corresponding to Israel Patent Application No. 104954]. Substitution of Gly^{14} by Asp resulted in a highly potent OGP antagonist (Table 3, Figure 7).

Table 2. Proliferative activity of OGP(10-14) analogs with L- or D-Ala substitutions

	Analog	Relative *in vitro* potency (95% confidence limit)	
		MC3T3 E1 cells	**NIH 3T3 cells**
1	OGP(1-14)	1.00 (standard)	1.00 (standard)
2	[Ala^{11}]OGP(11-14)	0.17(0.12-0.23)	0.07(0.03-0.12)
3	[Ala^{13}]OGP(11-14)	0.22(0.14-0.29)	0.10(0.05-0.15)
4	[Ala^{14}]OGP(11-14)	0.17(0.12-0.23)	0.10(0.06-0.13)
5	[Ala^{10}]OGP(10-14)	0.29(0.19-0.39)	0.17(0.04-0.30)
6	[Ala^{11}]OGP(10-14)	0.18(0.13-0.22)	0.31(0.24-0.37)
7	desamino[Ala^{10}]OGP(10-14)	0.28(0.07-0.49)	0.09(0.00-0.18)
8	desamino[Tyr^{10}, Ala^{11}]OGP(10-14)	0.41(0.29-0.53)	0.43(0.38-0.48)
9	desamino[Tyr^{10}, Ala^{12}]OGP(10-14)	0.21(0.12-0.30)	0.16(0.06-0.26)
10	desamino[Tyr^{10}, Ala^{13}]OGP(10-14)	0.27(0.23-0.31)	0.15(0.09-0.21)
11	desamino[Tyr^{10}, [Ala^{14}]OGP(10-14)	0.19(0.04-0.34)	0.16(0.06-0.26)
12	[D-Ala^{10}]OGP(10-14)	0.12(0.00-0.25)	0.16(0.05-0.27)
13	[D-Ala^{13}]OGP(10-14)	0.14(0.13-0.16)	0.26(0.20-0.31)
14	desamino[Tyr^{10},D-Ala^{11}]OGP(10-14)	0.21(0.00-0.55)	0.19(0.09-0.29)
15	desamino[Tyr^{10},D-Ala^{12}]OGP(10-14)	0.30(0.13-0.47)	0.02(0.00-0.06)
16	desamino[Tyr^{10},D-Ala^{13}]OGP(10-14)	0.28(0.19-0.37)	0.23(0.12-0.34)
17	desamino[Tyr^{10},D-Ala^{14}]OGP(10-14)	0.41(0.27-0.55)	0.32(0.17-0.47)

Table 3. Proliferative activity of OGP(10-14) analogs with Phe substitution of Tyr^{10}

Analog		Relative *in vitro* potency (95% confidence limit)	
		MC3T3 E1 cells	**NIH 3T3 cells**
1	OGP(1-14)	1.00 (standard)	1.00 (standard)
2	$[Phe^{10}]$OGP(10-14)	0.41(0.27-0.55)	0.32(0.17-0.47)
3	desamino$[Phe^{10}]$OGP(10-14)	0.35(0.28-0.42)	0.48(0.42-0.54)
4	(desamino$[Phe^{10}])_2$OGP(10-14)	0.18(0.15-0.22)	0.24(0.14-0.33)

Table 4. Proliferative activity of OGP(10-14) analogs with modifications at position 11 and 14

Analog		Relative *in vitro* potency (95% confidence limit)	
		MC3T3 E1 cells	**NIH 3T3 cells**
1	OGP(1-14)	1.00 (standard)	1.00 (standard)
2	des$[Gly^{11}]$OGP(10-14)	0.21(0.17-0.25)	0.17(0.11-0.23)
3	$[\beta\text{-}Ala^{11}]$OGP(10-14)	0.29(0.24-0.34)	0.17(0.13-0.21)
4	$[Asp^{14}]$OGP(10-14)	-0.39(-0.26--0.52)	-0.28(-0.14--0.42)

Most of the structurally constrained OGP analogs show similar or improved activity as compared to the full length OGP. The activity remained essentially unaltered following replacement of Gly^{11} by Pro (Table 5, analog 2). Rigidification of the OGP(10-14) structure by cyclization also preserved or slightly improved its *in vitro* activity as demonstrated by the analogs c(Tyr-Gly-Phe-Gly-Gly) (Table 5, analog 7), c(Gly-Gly-Phe-Gly-Tyr) (Table 5, analog 9) and c(Gly-Gly-D-Phe-Gly-D-Tyr) (Table 5, analog 11) (Figure 2). c(D-Tyr-Gly-D-Phe-Gly-Gly) (Table 5, analog 10) also retained a considerable level of proliferative activity. In addition, the *in vivo* activity of c(Tyr-Gly-Phe-Gly-Gly) (Table 5, analog 7), i.e. reversal of the OVX induced reduction in bone marrow derived osteoprogenitor cells and osteoblastic colonies, was improved over OGP(10-14) (Figure 6). The introduction of constraints which may alter the Tyr/Phe relationship resulted in less active, or in many instances almost inactive, OGP analogs. Structurally constrained peptide-based drugs usually present improved efficacy as a consequence of their increased (i) resistance to peptidase degradation and longer persistence in the

circulation; (ii) potency and thus improved cellular responsiveness; (iii) bioavailability through non-parenteral routes, e.g. oral.

Table 5. Proliferative activity of constrained OGP analogs

	Analog	Relative *in vitro* potency (95% confide limit)	
		MC3T3 E1 cells	**NIH 3T3 cells**
1	OGP(1-14)	1.00 (standard)	1.00 (standard)
2	[Pro^{11}]OGP(10-14)	0.89(0.80-0.98)	0.96(0.87-1.05)
3	desamino[Tyr^{10},Sar^{11}]OGP(10-14)	0.31(0.25-0.37)	0.39(0.26-0.52)
4	desamino[Tyr^{10},N(Me)-Phe^{12}]OGP(10-14)	0.52(0.46-0.58)	0.67(0.55-0.70)
5	desamino[Tyr^{10},Sar^{13}]OGP(10-14)	0.15(0.07-0.23)	0.11(0.05-0.17)
6	desamino[Tyr^{10},Sar^{14}]OGP(10-14)	0.16(0.10-0.22)	0.14(0.09-0.19)
7	c(Tyr-Gly-Phe-Gly-Gly)	0.79(0.72-0.86)	1.12(1.06-1.17)
8	c(Tyr-Gly-Phe-Gly)	0.35(0.30-0.40)	0.43(0.40-0.46)
9	c(Gly-Gly-Phe-Gly-Tyr)	0.95(0.89-1.01)	1.02(0.93-1.11)
10	c(D-Tyr-Gly-D-Phe-Gly-Gly)	0.69(.62-0.76)	0.84(0.80-0.88)
11	c(Gly-Gly-D-Phe-Gly-D-Tyr)	1.03(0.95-1.11)	1.16(1.10-1.22)
12	c(Gly-Tyr-Gly-Phe-Gly-Gly)	0.26(0.19-0.33)	0.20(0.17-0.23)
13	c(β-Ala-Tyr-Gly-Phe-Gly-Gly)	0.36(0.30-0.42)	0.37(0.31-0.43)
14	c(γ-Abu-Tyr-Gly-Phe-Gly-Gly)	0.20(0.16-0.24)	0.22(0.19-0.25)
15	c(δ-Ala-Tyr-Gly-Phe-Gly-Gly)	0.14(0.09-0.19)	0.18(0.13-0.23)
16	c(Tyr-Gly-Phe-Gly-Asp)-OH	0.14(0.09-0.19)	0.11(0.07-0.15)
17	c(Gly-Tyr-Gly-Phe-Gly-Asp)-OH	0.15(0.11-0.19)	0.16(0.12-0.20)
18	c(β-Ala-Tyr-Gly-Phe-Gly-Asp)-OH	-0.08(-0.04--0.12)	-0.19(-0.15--0.23)
19	c(γ-Abu-Tyr-Gly-Phe-Gly-Asp)-OH	0.13(0.10-0.16)	0.07(0.03-0.11)
20	c(δ-Ala-Tyr-Gly-Phe-Gly-Asp)-OH	0.20(0.14-0.26)	0.11(0.09-0.13)

The following pseudopeptide analogs of OGP(10-14): desamino[Tyr$^{10}\psi$ (CH_2NH)Gly^{11}]OGP(10-14) (Table 6, analog 2), desamino[Tyr^{10},Gly$^{11}\psi$ (CH_2NH)Phe^{12}]OGP(10-14) (Table 6, analog 3), desamino[Tyr^{10},Phe$^{12}\psi$

(CH_2NH)Gly^{13}]OGP(10-14) (Table 6, analog 4), desamino[$Tyr^{10}Gly^{13}\psi$(CH_2NH)Gly^{14}]OGP(10-14) (Table 6, analog 5), desamino[$Tyr^{10}Gly^{13}\psi$(CH_2)$_2Gly^{14}$]OGP(10-14) (Table 6, analog 6), had a similar or improved activity compared to desamino[Tyr^{10}]OGP(10-14) (Table 1, analog 4) also because of increased resistance to peptidase degradation.

Table 6. Proliferative activity of non-constrained pseudopeptide OGP analogs

	Analog	Relative *in vitro* potency (95% confidence limit)	
		MC3T3 E1 cells	NIH 3T3 cells
1	OGP(1-14)	1.00 (standard)	1.00 (standard)
2	desamino[$Tyr^{10}\psi$(CH_2NH)Gly^{11}]OGP(10-14)	0.81(0.71-0.91)	0.79(0.67-0.91)
3	desamino[Tyr^{10},$Gly^{11}\psi$(CH_2NH)Phe^{12}]OGP(10-14)	0.61(0.53-0.69)	0.67 (0.60-0.74)
4	desamino[Tyr^{10},$Phe^{12}\psi$(CH_2NH)Gly^{13}]OGP(10-14)	0.70(0.65-0.75)	0.88(0.76-1.00)
5	desamino[$Tyr^{10}Gly^{13}\psi$(CH_2NH)Gly^{14}]OGP(10-14)	0.78(0.73-0.83)	0.80(0.67-0.93)
6	desamino[$Tyr^{10}Gly^{13}\psi$(CH_2)$_2Gly^{14}$]OGP(10-14)	0.78(0.73-0.83)	0.88(0.79-0.97)
7	[$Gly^{13}\psi$(CH_2)$_2Gly^{14}$]OGP(11-14)	0.15(0.11-0.19)	0.08(0.05-0.13)
8	N(Me)-[Tyr^{10}]OGP(9-14)	-0.34(-0.19--0.49)	-0.32(-0.27--0.37)
9	N(Me)-[Tyr^{10}]OGP(1-14)	0.34(0.27-0.41)	0.42(0.35-0.49)
10	[$Leu^9\psi$(CH_2NH)Tyr^{10}]OGP(1-14)	0.45(0.41-0.49)	0.31(0.29-0.33)

Since OGP(10-14) is a naturally occurring peptide [WO94/20529 corresponding to Israel Patent Application No. 104954] the dependence of the OGP(1-14) mitogenic activity on OGP(10-14) formation by proteolysis was assessed using the analogs [N(Me)-Tyr^{10}]OGP(1-14) (Table 6, analog 9) and [$Leu^9\psi$(CH_2NH)Tyr^{10}]OGP(1-14) (Table 6, analog 10). Either substitution of the natural peptide bond between Leu^9 and Tyr^{10} resulted in more than 50% inhibition of the OGP(1-14) activity (Table 6, Figure 3), suggesting that OGP(10-14) is essential for the full OGP-like activity. However, truncation of the eight N-terminal amino acid residues of one of these analogs yielded another highly potent OGP antagonist, [N(Me)-Tyr^{10}]OGP(9-14) (Table 6, analog 8) (Figure 7). In the absence of exogenous OGP both antagonists, [N(Me)-Tyr^{10}]OGP(9-14) and [Asp^{14}]OGP(10-14), inhibit osteoblastic MC3T3 E1 cell proliferation dose dependently at low concentrations with reversal of this inhibition at high doses. The analog concentration evoking the

peak inhibitory response is 10^{-13} M (Figure 8). The peak stimulatory response to OGP is seen at the same peptide dose [Bab, I., *et al.* (1992) EMBO J. 11:1867; Greenberg, Z., *et al* (1993) Biochim Biophys Acta 1178:273; Greenberg, Z., *et al* (1995) J. Clin. Endocrinol. Metab 80:2330; U.S. Patent No. 5,461,034]. This dose-response pattern suggests that [N(Me)-Tyr^{10}]OGP(9-14) and [Asp^{14}]OGP(10-14) antagonize not only the effect of exogenously administered OGP but also the regulatory action of endogenous OGP [Bab, I., *et al.* (1992) EMBO J. 11:1867; Greenberg, Z., *et al* (1995) J. Clin. Endocrinol. Metab 80: 2330] and may therefore be used to neutralize undesirable OGP-like responses particularly in instances characterized by excess endogenous OGP.

A benzoyl was introduced in position 4 of the Phe^{12} aromatic ring (Table 7, analog 2) to assess the feasibility of photoaffinity crosslinking of an OGP probe to the putative OGP receptor. This modification had only a minor effect on the OGP-like proliferative activity (Figure 4). This activity remained unaltered following iodination of Tyr^{10} or addition of a biotinylcaproyl group to the N-terminal of [Bpa^{12}]OGP(10-14) (Table 7, Figure 4), suggesting that either analog, [Tyr^{10}(m-I),Bpa^{12}]OGP(10-14) or Nα-biotinylcaproyl-[Bpa^{12}]OGP(10-14), is a useful tagged, photoreactive ligand.

Table 7. Proliferative activity of labeled and/or photoreactive OGP(10-14) analogs

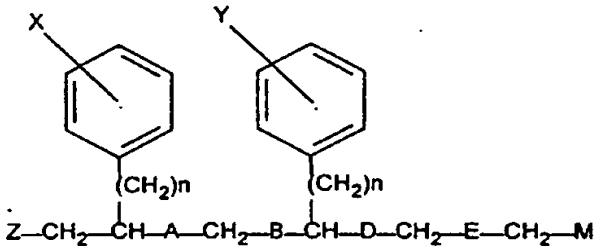
	Analog	Relative *in vitro* potency (95% confidence limit)	
		MC3T3 E1 cells	NIH 3T3 cells
1	OGP(1-14)	1.00 (standard)	1.00 (standard)
2	[Bpa^{12}]OGP(10-14)*	0.74(0.66-0.83)	0.86(0.75-0.97)
3	[Tyr^{10}(m-I),Bpa^{12}]OGP(10-14)	0.80(0.74-0.86)	0.85(0.76-0.94)
4	Nα-biotinylcaproyl-[Bpa^{12}]OGP(10-14) **		

* See Figure 4 for dose response curve.

**Tested once in triplicate culture wells - see Figure 4 for dose response curve.

CLAIMS:

1. Pseudopeptidic osteogenic growth polypeptide (OGP) analogs having the general formula:



(I)

wherein

D and E, which may be the same or different, represent CONH, CH_2NH, CH_2S, CH_2O, NHCO, $N(CH_3)CO$, $(CH_2)_2$, CH=CH, $C(O)CH_2$, CH_2SO or C(O)O,

M represents C(O)OH, CH_2OH, $C(O)NH_2$, $C(O)OCH_3$, CH_2OCH_3, H, $C(O)NHCH_3$, or $C(O)N(CH_3)_2$,

Z represents NH_2, H, $NHCH_3$, $N(CH_3)_2$, OH, SH, OCH_3, SCH_3. C(O)OH, $C(O)NH_2$, $C(O)OCH_3$, $C(O)NHCH_3$ or $C(O)N(CH_3)_2$,

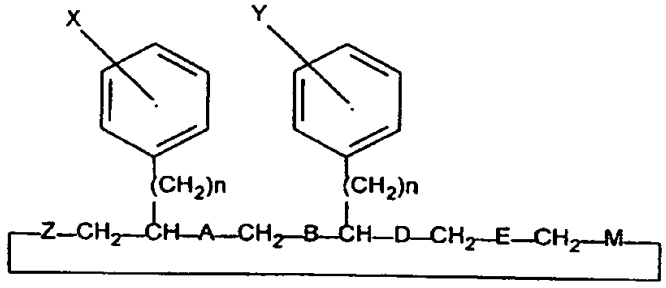
n and m each represent an integer of from 1 to 6,

X and Y, if in the ortho or para positions, each represent OH, OCH_3, F, Cl, Br, CF_3, CN, NO_2, NH_2, $NHCH_3$, $N(CH_3)_2$, SH, SCH_3, CH_2OH, $NHC(O)CH_3$, C(O)OH, $C(O)OCH_3$, $C(O)NH_2$, $C(O)NHCH_3$, $C(O)N(CH_3)_2$, or CH_3, and

if in the para or meta positions, represents $C(O)C_6H_5$, $C(O)CH_3$, C_6H_5, $CH_2C_6H_5$, and, if in the ortho or para positions can additionally represent $C(O)C_6H_5$, $C(O)CH_3$, C_6H_5, $CH_2\ C_6H_5$, CH_2CH_3, $CH(CH_3)\ 2$, or C_6H_{11}.

2. Pseudopeptidic OGP analogs having the general formula:

20

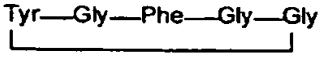


wherein Z — M represent NHC(O), C(O)NH, CH_2NH, NH_2CH_2, $N(CH_3)C(O)$, $C(O)N(CH_3)$, C(O)O, OC(O), OR $(CH_2)_l$ where l is an integer of from 2 to 6 and A, B, D, E, n, m, X and Y are as defined in claim 1.

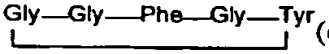
3. A pseudopeptidic OGP analog according to claim 1 being:

desaminoTyr-Gly-Phe-Gly-Gly (desamino[Tyr^{10}]OGP(10-14) as hereinbefore defined), desaminoTyr-Gly-$N(CH_3)$-$CH(CH_2C_6H_5)$-C(O)-Gly-Gly (desamino[Tyr^{10},N(Me)-Phe^{12}]OGP(10-14) as hereinbefore defined), desamino$CH(CH_2C_6H_5OH)$-CH_2-Gly-Phe-Gly-Gly (desamino[$Tyr^{10}\psi$ $(CH_2NH)Gly$]OGP(10-14) as hereinbefore defined), desaminoTyr-NH-CH_2-CH_2-Phe-Gly-Gly, (desamino[Tyr^{10},$Gly^{11}\psi(CH_2NH)Phe^{12}$]OGP(10-14) as hereinbefore defined), desaminoTyr-Gly-NH-$CH(CH_2C_6H_5)$-CH_2-Gly-Gly (desamino[Tyr^{10},$Phe^{12}\psi(CH_2NH)$-Gly^{13}]OGP(10-14) as hereinbefore defined), desaminoTyr-Gly-Phe-NH-CH_2-CH_2-Gly (desamino[Tyr^{10},$Gly^{13}\psi$ $(CH_2NH)Gly^{14}$]OGP(10-14) as hereinbefore defined), desaminoTyr-Gly-Phe-NH-CH_2-CH_2CH_2-CH_2-C(O)-OH (desamino[Tyr^{10},$Gly^{13}\psi$ $(CH_2)_2Gly^{14}$]OGP(10-14) as hereinbefore defined), Tyr-Gly-NH-$CH(CH_2C_6H_4$-(C(O)-C_6H_5))-C(O)-Gly-Gly ([Bpa^{12}] OGP(10-14) as hereinbefore defined), Tyr(m-I)-Gly-NH-$CH(CH_2C_6H_4(C(O)C_6H_5))$C(O)-Gly-Gly ([Tyr(m-I),$Bpa^{12}$]OGP(10-14) as hereinbefore defined) or Nα-biotinylcaproyl-[Bpa^{12}]OGP(10-14) as hereinbefore defined.

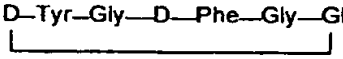
4. A peptidic or pseudopeptidic OGP analog according to claim 2 being:



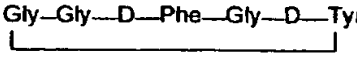
(c[Tyr-Gly-Phe-Gly-Gly] as herein before defined),



(c[Gly-Gly-Phe-Gly-Tyr] as hereinbefore defined),



(c[D-Tyr-Gly-D-Phe-Gly-Gly] as hereinbefore defined) or



(c[Gly-Gly-D-Phe-Gly-D-Tyr]as hereinbefore defined).

5. Pseudopeptidic osteogenic growth factor antagonists being Leu-N(CH_3)-CH($CH_2C_6H_4$(OH))-C(O)-Gly-Phe-Gly-Gly ([N(CH_3)Tyr^{10}]OGP(9-14) as hereinbefore defined) and Tyr-Gly-Phe-Gly-Asp ([Asp^{14}]OGP(10-14) as hereinbefore defined).

6. Pharmaceutical composition comprising as active ingredient at least one pseudopeptide of formula (I), optionally with a pharmaceutically acceptable carrier.

7. Pharmaceutical composition according to claim 6 wherein said pseudopeptide is desamino[Tyr^{10}]OGP(10-14).

8. Pharmaceutical composition comprising as active ingredient at least one cyclic peptide or pseudopeptide of formula (II), optionally with a pharmaceutically acceptable carrier.

9. Pharmaceutical composition according to claim 8 wherein said cyclic peptide is c[Tyr-Gly-Phe-Gly-Gly].

10. Pharmaceutical composition comprising as active ingredient at least one pseudopeptide of formula (I) and at least one cyclic peptide or pseudopeptide of formula (II),optionally with a pharmaceutically acceptable carrier.

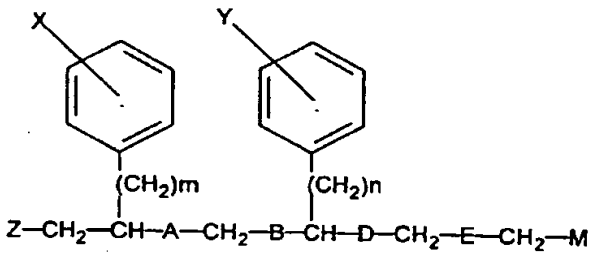
11. A pseudopeptide according to claim 1 or claim 3 for use in the preparation of a pharmaceutical composition for stimulating the formation of osteoblastic or fibroblastic cells, enhancing bone formation in osteopenic pathological conditions, repairing fractures, healing wounds, grafting of intraosseous implants, reversing bone loss in osteoporosis and other conditions requiring enhanced bone cells formation.

12. A cyclic peptide or pseudopeptide according to claim 2 or claim 4 for use in the preparation of a pharmaceutical composition for stimulating the formation of osteoblastic or fibroblastic cells, enhancing bone formation in osteopenic pathological conditions, repairing fractures, healing wounds, grafting of intraosseous implants, reversing bone loss in osteoporosis and other conditions requiring enhanced bone cells formation.

AMENDED CLAIMS

[received by the International Bureau on 28 July 1997 (28.07.97); original claims 1 and 2 amended; remaining claims unchanged (3 pages)]

1. Pseudopeptidic osteogenic growth polypeptide (OGP) analogs having the general formula:



(I)

wherein

A, B, D and E, which may be the same or different, represent CONH, CH_2NH, CH_2S, CH_2O, NHCO, $N(CH_3)CO$, $(CH_2)_2$, CH=CH, $C(O)CH_2$, CH_2SO or C(O)O,

M represents C(O)OH, CH_2OH, $C(O)NH_2$, $C(O)OCH_3$, CH_2OCH_3, H, $C(O)NHCH_3$, or $C(O)N(CH_3)_2$,

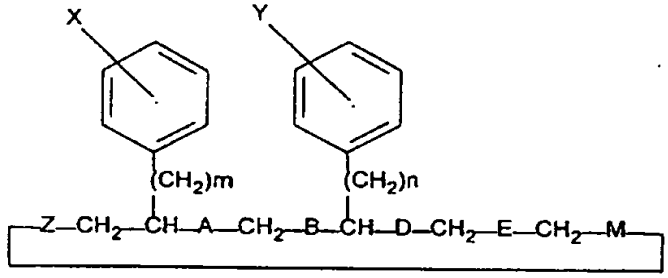
Z represents NH_2, H, $NHCH_3$, $N(CH_3)_2$, OH, SH, OCH_3, SCH_3. C(O)OH, $C(O)NH_2$, $C(O)OCH_3$, $C(O)NHCH_3$ or $C(O)N(CH_3)_2$,

n and m each represent an integer of from 1 to 6,

X and Y, if in the ortho or para positions, each represent OH, OCH_3, F, Cl, Br, CF_3, CN, NO_2, NH_2, $NHCH_3$, $N(CH_3)_2$, SH, SCH_3, CH_2OH, $NHC(O)CH_3$, C(O)OH, $C(O)OCH_3$, $C(O)NH_2$, $C(O)NHCH_3$, $C(O)N(CH_3)_2$, or CH_3, and

if in the para or meta positions, represents $C(O)C_6H_5$, $C(O)CH_3$, C_6H_5, $CH_2C_6H_5$, and, if in the ortho or para positions can additionally represent $C(O)C_6H_5$, $C(O)CH_3$, C_6H_5, $CH_2\ C_6H_5$, CH_2CH_3, $CH(CH_3)_2$, or C_6H_{11}.

2. Pseudopeptidic OGP analogs having the general formula:



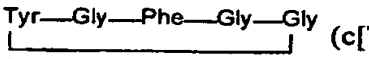
(II)

wherein Z — M represent NHC(O), C(O)NH, CH_2NH, NH_2CH_2, $N(CH_3)C(O)$, $C(O)N(CH_3)$, C(O)O, OC(O), OR $(CH_2)_l$ where l is an integer of from 2 to 6 and A, B, D, E, n, m, X and Y are as defined in claim 1.

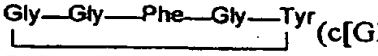
3. A pseudopeptidic OGP analog according to claim 1 being:

desaminoTyr-Gly-Phe-Gly-Gly (desamino[Tyr^{10}]OGP(10-14) as hereinbefore defined), desaminoTyr-Gly-$N(CH_3)$-$CH(CH_2C_6H_5)$-C(O)-Gly-Gly (desamino[Tyr^{10},N(Me)-Phe^{12}]OGP(10-14) as hereinbefore defined), desaminoCH($CH_2C_6H_5OH$)-CH_2-Gly-Phe-Gly-Gly (desamino[$Tyr^{10}\psi(CH_2NH)Gly$]OGP(10-14) as hereinbefore defined), desaminoTyr-NH-CH_2-CH_2-Phe-Gly-Gly, (desamino[Tyr^{10},$Gly^{11}\psi(CH_2NH)Phe^{12}$]OGP(10-14) as hereinbefore defined), desaminoTyr-Gly-NH-$CH(CH_2C_6H_5)$-CH_2-Gly-Gly (desamino[Tyr^{10},$Phe^{12}\psi(CH_2NH)$-Gly^{13}]OGP(10-14) as hereinbefore defined), desaminoTyr-Gly-Phe-NH-CH_2-CH_2-Gly (desamino[Tyr^{10},$Gly^{13}\psi(CH_2NH)Gly^{14}$]OGP(10-14) as hereinbefore defined), desaminoTyr-Gly-Phe-NH-CH_2-CH_2CH_2-CH_2-C(O)-OH (desamino[Tyr^{10},$Gly^{13}\psi(CH_2)_2Gly^{14}$]OGP(10-14) as hereinbefore defined), Tyr-Gly-NH-$CH(CH_2C_6H_4$-(C(O)-C_6H_5))-C(O)-Gly-Gly ([Bpa^{12}] OGP(10-14) as hereinbefore defined), Tyr(m-I)-Gly-NH-$CH(CH_2C_6H_4(C(O)C_6H_5))$C(O)-Gly-Gly ([Tyr(m-I),$Bpa^{12}$]OGP(10-14) as hereinbefore defined) or Nα-biotinylcaproyl-[Bpa^{12}]OGP(10-14) as hereinbefore defined.

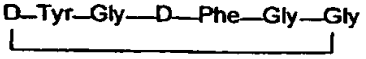
4. A peptidic or pseudopeptidic OGP analog according to claim 2 being:



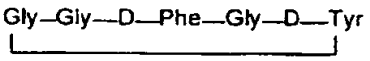
(c[Tyr-Gly-Phe-Gly-Gly] as herein before defined),



(c[Gly-Gly-Phe-Gly-Tyr] as hereinbefore defined),



(c[D-Tyr-Gly-D-Phe-Gly-Gly] as hereinbefore

defined) or  (c[Gly-Gly-D-Phe-Gly-D-Tyr]as hereinbefore defined).

5. Pseudopeptidic osteogenic growth factor antagonists being Leu-N(CH_3)-CH($CH_2C_6H_4$(OH))-C(O)-Gly-Phe-Gly-Gly ([N(CH_3)Tyr^{10}]OGP(9-14) as hereinbefore defined) and Tyr-Gly-Phe-Gly-Asp ([Asp^{14}]OGP(10-14) as hereinbefore defined).

6. Pharmaceutical composition comprising as active ingredient at least one pseudopeptide of formula (I), optionally with a pharmaceutically acceptable carrier.

7. Pharmaceutical composition according to claim 6 wherein said pseudopeptide is desamino[Tyr^{10}]OGP(10-14).

8. Pharmaceutical composition comprising as active ingredient at least one cyclic peptide or pseudopeptide of formula (II), optionally with a pharmaceutically acceptable carrier.

9. Pharmaceutical composition according to claim 8 wherein said cyclic peptide is c[Tyr-Gly-Phe-Gly-Gly].

10. Pharmaceutical composition comprising as active ingredient at least one pseudopeptide of formula (I) and at least one cyclic peptide or pseudopeptide of formula (II),optionally with a pharmaceutically acceptable carrier.

11. A pseudopeptide according to claim 1 or claim 3 for use in the preparation of a pharmaceutical composition for stimulating the formation of osteoblastic or fibroblastic cells, enhancing bone formation in osteopenic pathological conditions, repairing fractures, healing wounds, grafting of intraosseous implants, reversing bone loss in osteoporosis and other conditions requiring enhanced bone cells formation.

12. A cyclic peptide or pseudopeptide according to claim 2 or claim 4 for use in the preparation of a pharmaceutical composition for stimulating the formation of osteoblastic or fibroblastic cells, enhancing bone formation in osteopenic pathological conditions, repairing fractures, healing wounds, grafting of intraosseous implants, reversing bone loss in osteoporosis and other conditions requiring enhanced bone cells formation.

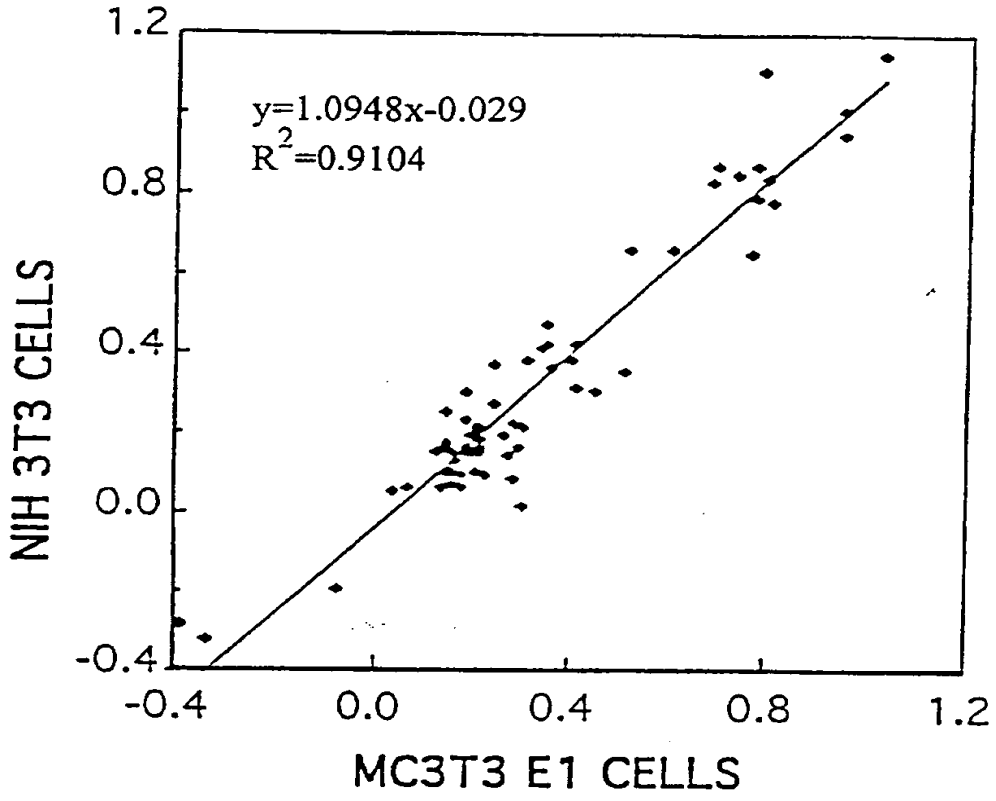


Figure 1

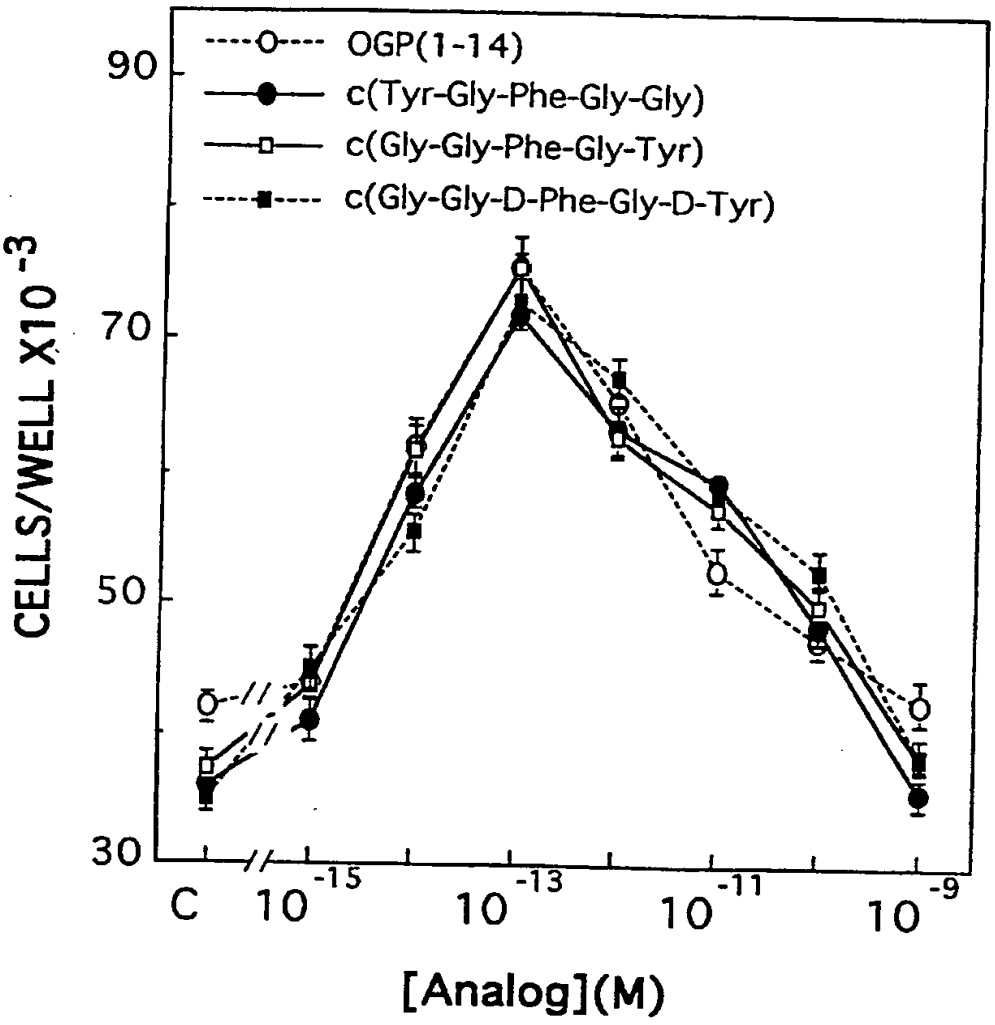


Figure 2

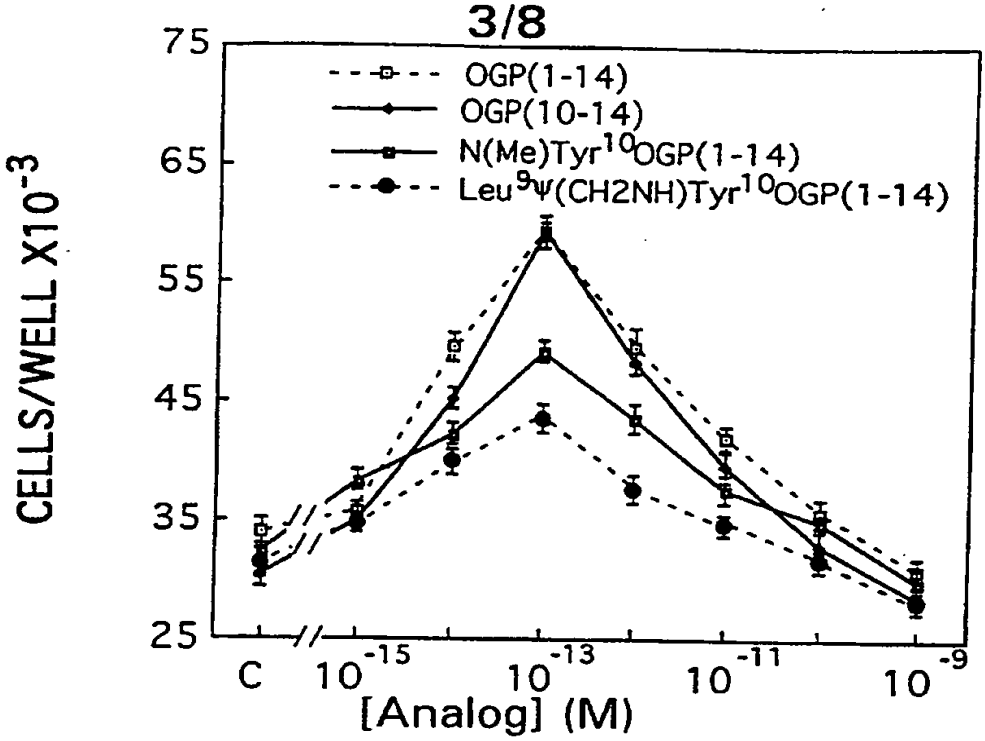


Figure 3A

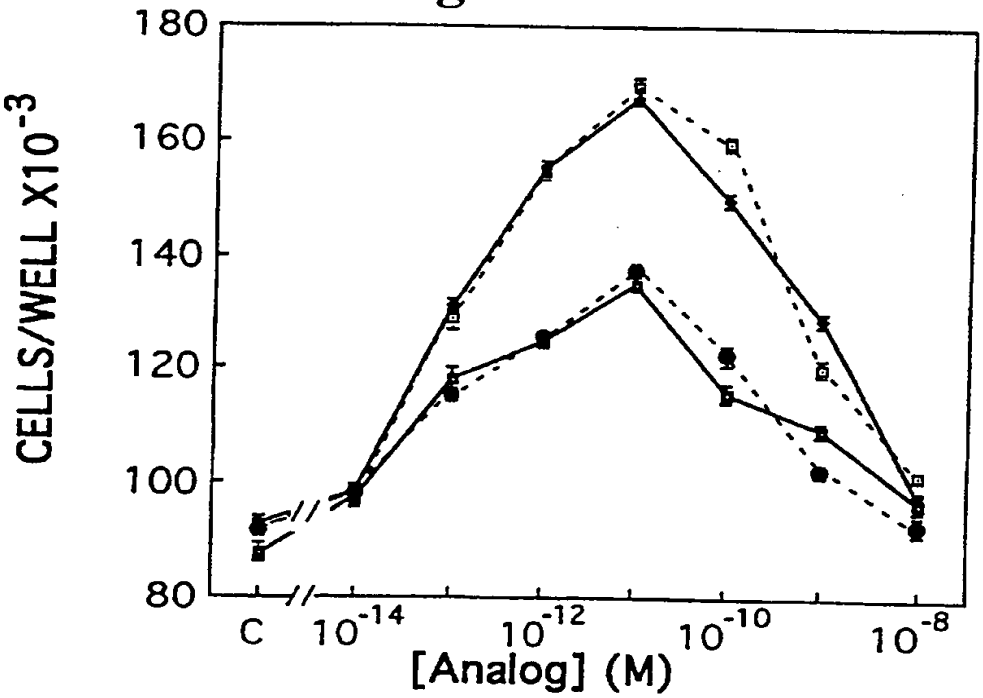


Figure 3B

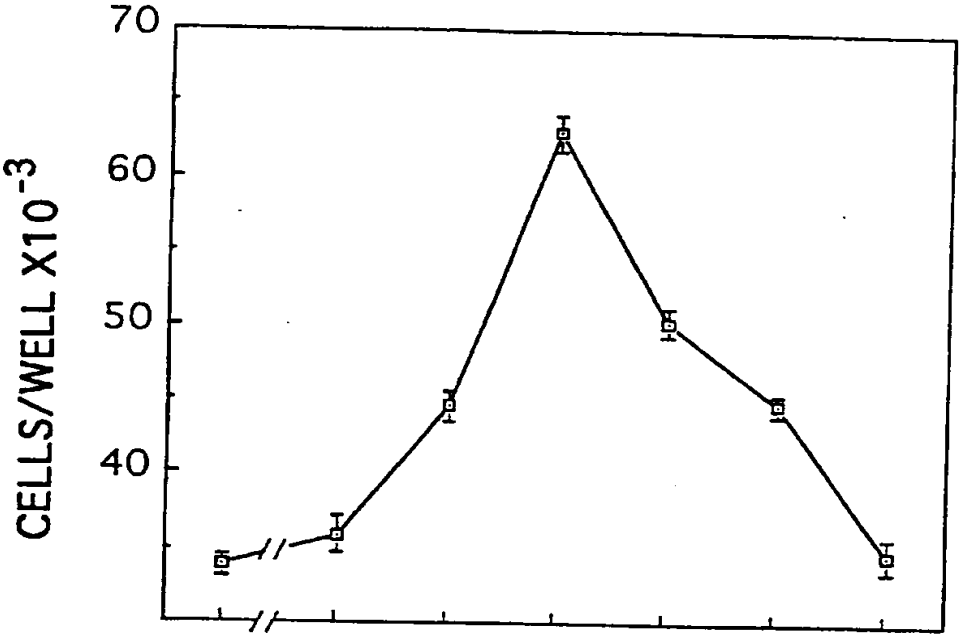


Figure 4A

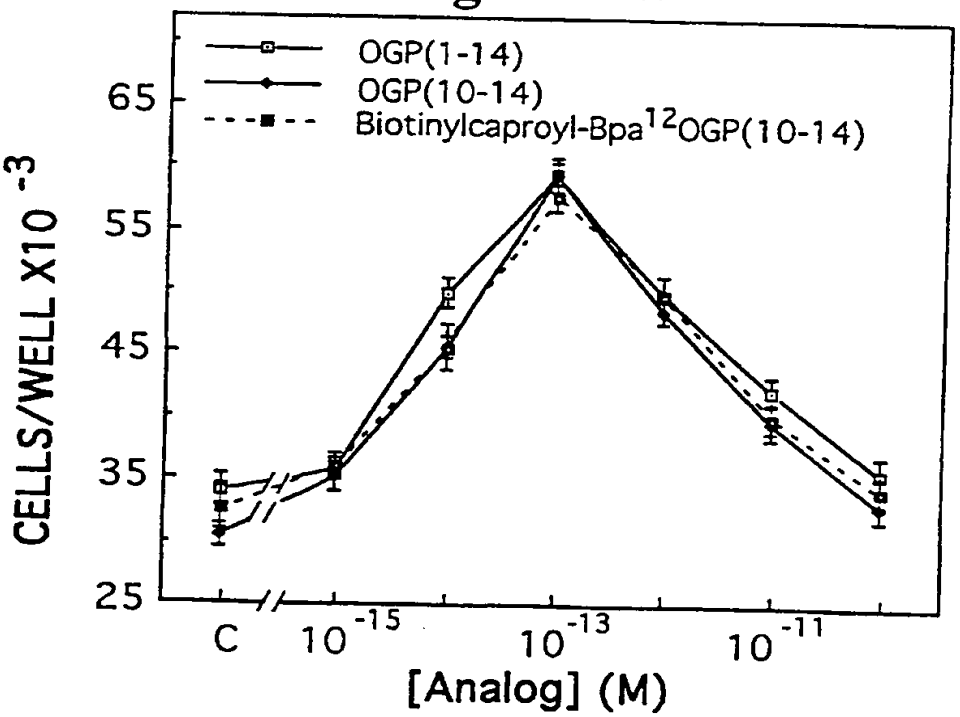


Figure 4B

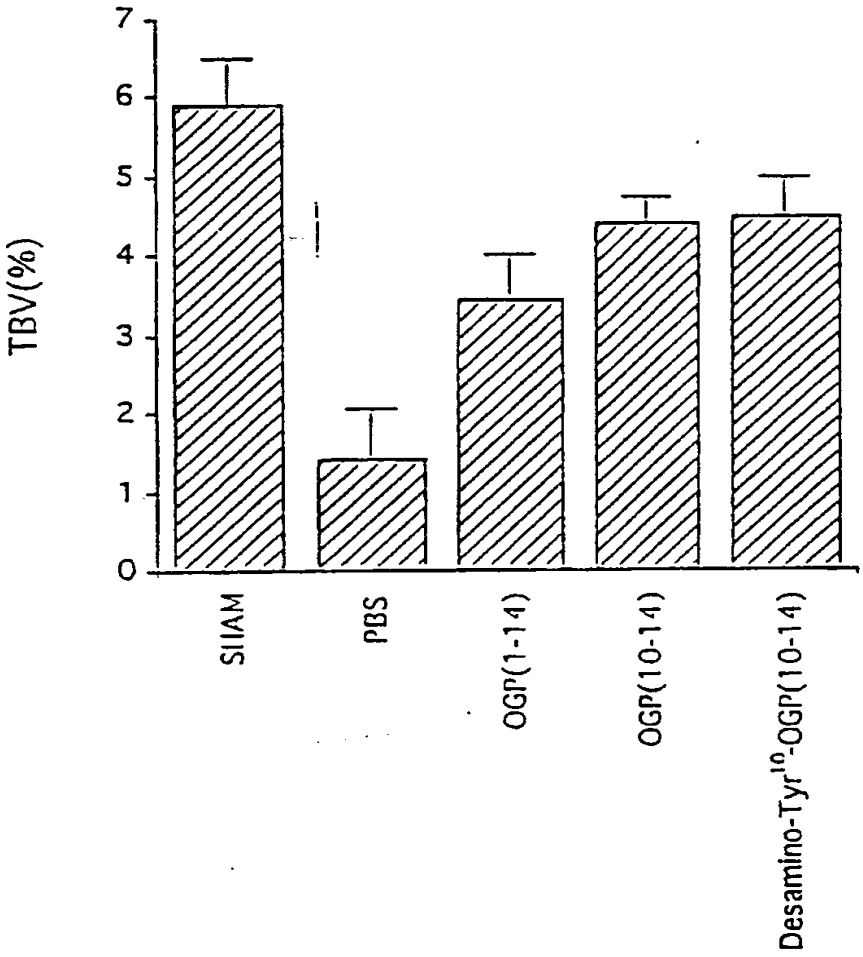

TBV(%)
0
1
2
3
4
5
6
7
SIIAM
PBS
OGP(1-14)
OGP(10-14)
Desamino-Tyr[10]-OGP(10-14)

Figure 5

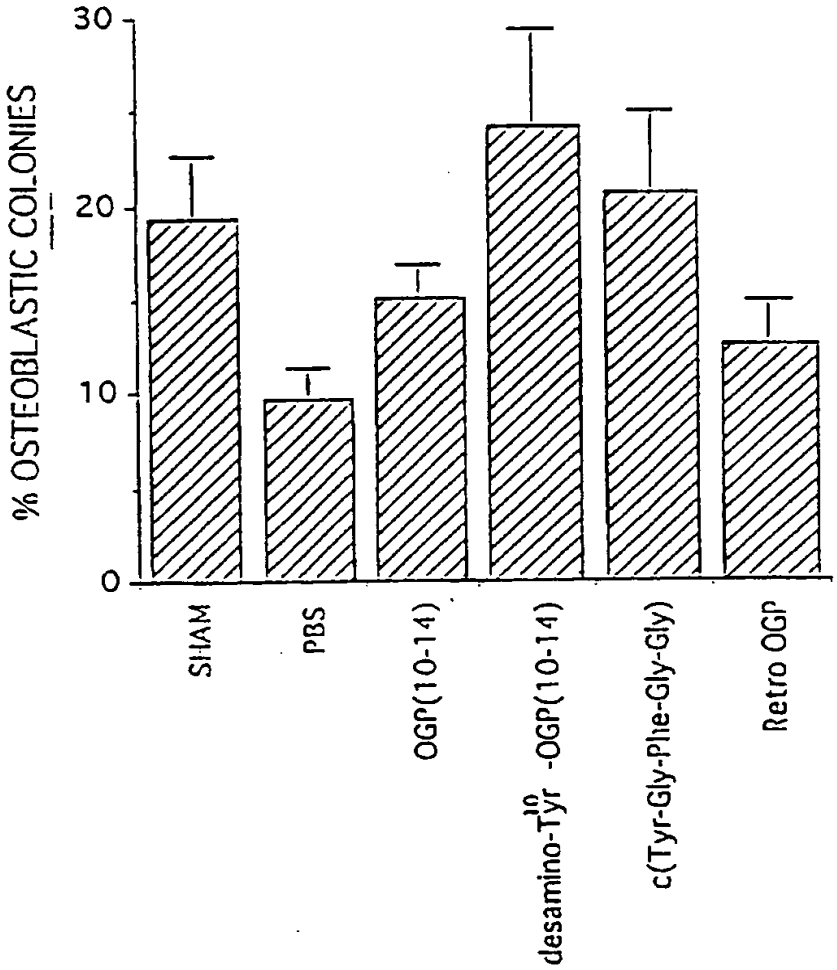

% OSTEOBLASTIC COLONIES
30
20
10
0
SHAM
PBS
OGP(10-14)
desamino-Tyr[10]-OGP(10-14)
c(Tyr-Gly-Phe-Gly-Gly)
Retro OGP

Figure 6

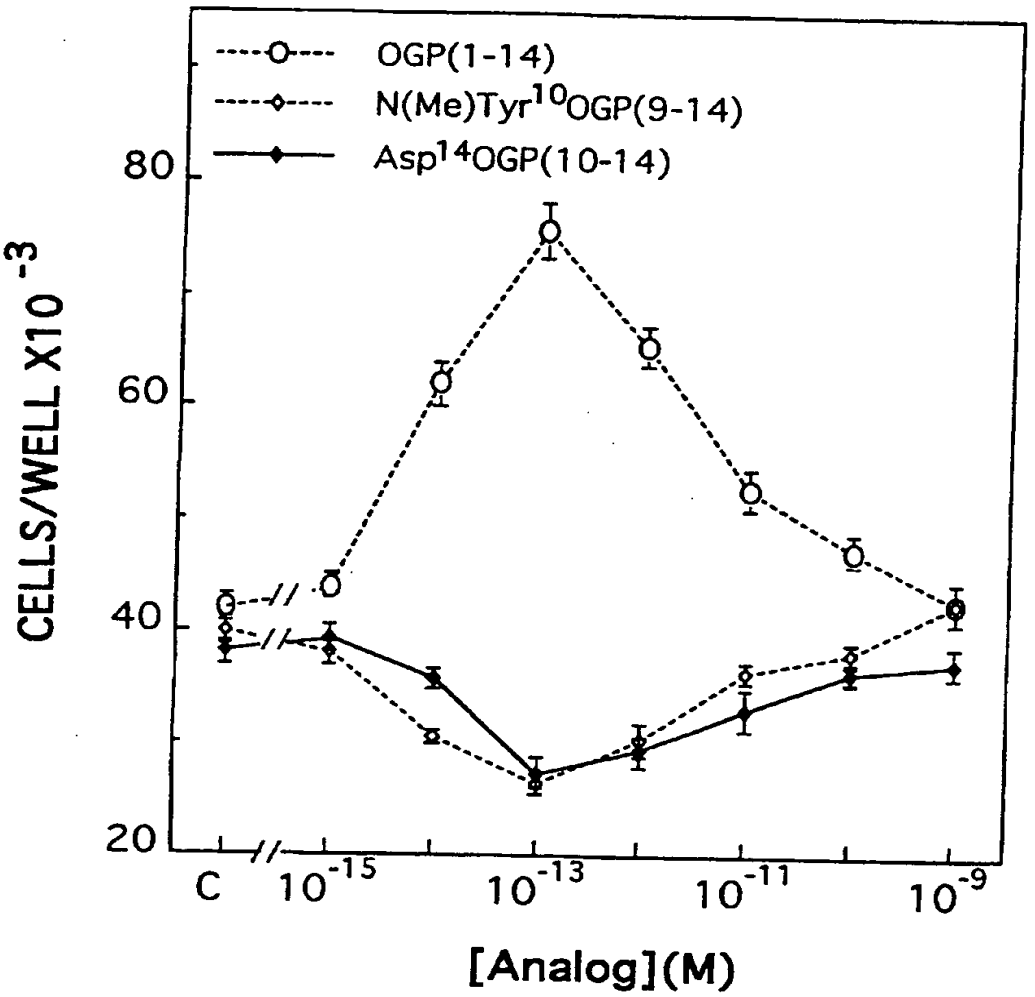


Figure 7

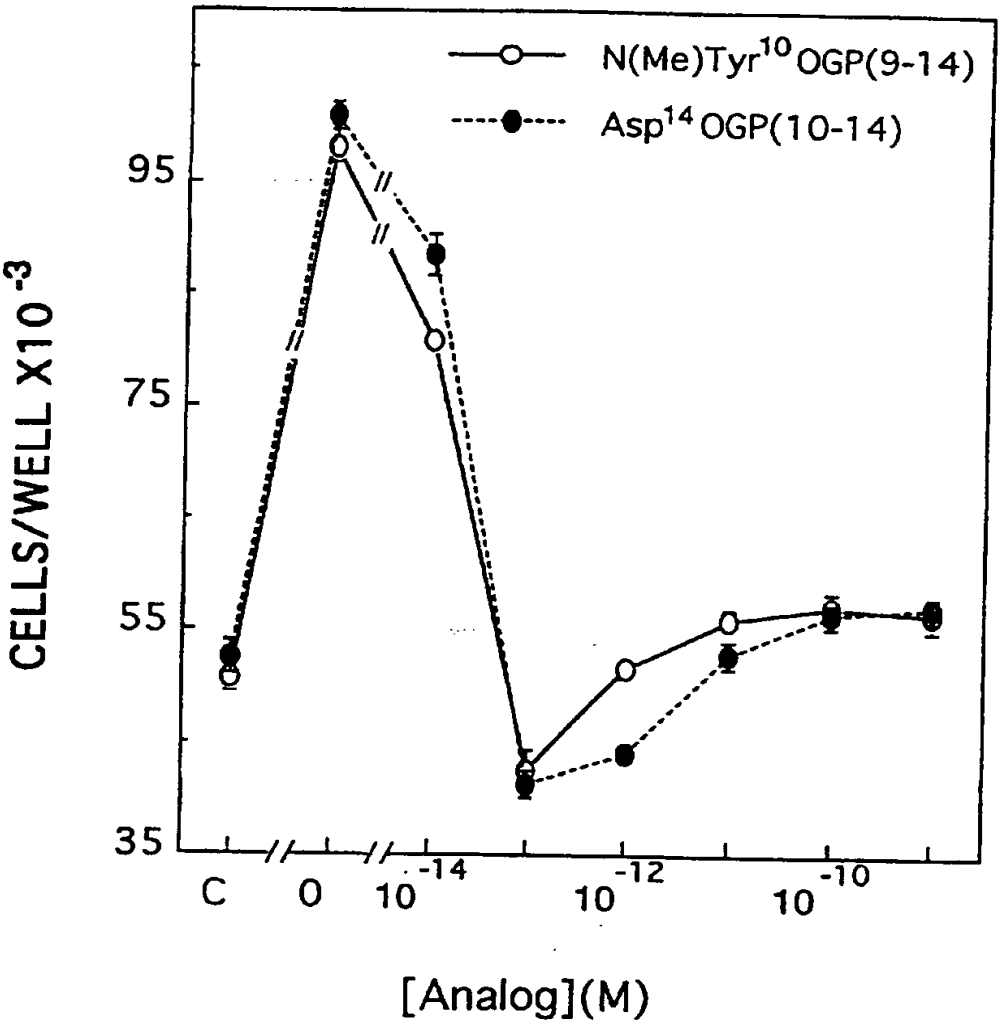


Figure 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL97/00087

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18; 530/300, 317, 324, 325, 326, 327, 328, 329, 330, 350, 402

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG (Biotech Files), GenEMBL sequence databases, STN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 572 122 A2 (YISSUM RESEARCH AND DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM) 01 December 1993, see entire document.	3-5, 7, 9
A	WO 94/20529 A1 (YISSUM RESEARCH ADN DEVELOPMENT COMPANY) 15 September 1994, see entire document.	3-5, 7, 9
A	BAB et al. Histone H4-related osteogenic growth peptide (OGP): a novel circulating stimulator of osteoblastic activity. The EMBO Journal. 1992, Vol. 11, No. 5, pages 1867-1873, see entire document.	3-5, 7, 9

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 23 JUNE 1997	Date of mailing of the international search report 09 JUL 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ELIZABETH C. KEMMERER Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL97/00087

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GREENBERG et al. Mitogenic action of osteogenic growth peptide (OGP): role of amino and carboxy-terminal regions and charge. Biochimica et Biophysica Acta. 1993, Vol. 1178, pages 273-280, see entire document.	3-5, 7, 9
A	GREENBERG et al. Structural and Functional Characterization of Osteogenic Growth Peptide from Human Serum: Identity with Rat and Mouse Homologs. Journal of Clinical Endocrinology and Metabolism. Vol. 80, No. 8, 1995, pages 2330-2335, see entire document.	3-5, 7, 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL97/00087

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1, 2, 6, 8, and 10-12
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

There is no "m" in formula (I) or (II). Also, "A" and "B" appearing in formulas (I) and (II) are not defined in the claims.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL97/00087

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 38/03, 38/04, 38/08, 38/10, 38/12, 38/16; C07K 5/00, 5/12, 7/00, 7/64, 11/00, 11/02, 14/00

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

514/2, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18; 530/300, 317, 324, 325, 326, 327, 328, 329, 330, 350, 402